REMARKS

By this amendment, Applicants have corrected several minor informalities in the Sequence Listing and specification.

The Sequence Listing has been amended to more accurately describe what is disclosed in the application. For example, SEQ ID NO:4 has been amended to include the amino acids Met Gly Lys Tyr Met Lys, in sequential order, at the N-terminal end. These amino acids are clearly visible in Figure 1 as originally filed. No new matter has been introduced into the application. Further with respect to Figure 1, Applicants also submit the second page of same which inadvertently, may not have been submitted with the original application when filed. The present application is a continuation-in-part application under 35 U.S.C. 120 of U.S. Serial No. 09/526,597, filed March 16, 2000. The parent application, Serial No. 09/526,597 has two pages to Figure 1: (1/2) and (2/2). Thus, the submission of the second page (2/2) in the present application does not introduce new matter into the application as originally filed. Entry of the second page of Figure 1 is therefore respectfully requested.

In the Sequence Listing submitted in the Preliminary Amendment on June 20, 2001, as well as this amendment, the nucleotide sequence for Sequence 18 has been changed. As originally filed, the sequences set forth in the Sequence Listing as SEQ ID NO:18 was identical to SEQ ID NO:17. Support for SEQ ID NO:18 in the Sequence Listing submitted June 20, 2001 and herewith may be found on page 93 of the specification and no new matter has been introduced.

The Sequence for SEQ ID NO:25 as originally filed was erroneous. SEQ ID NO:25, filed on June 20, 2001 as well as herewith as part of this amendment, reflects the sequence identified as SEQ ID NO:25 on page 97 of the specification. No new matter has been introduced to the application as originally filed.

As originally filed, the sequence set forth as SEQ ID NO:19 of the Sequence Listing was actually SEQ ID NO:18 as indicated in the text of the specification. In the Sequence Listing filed June 20, 2001 and well as herewith, SEQ ID NO:18 as set forth in the Sequence Listing reflects SEQ ID NO:18 as described on page 93 of the specification. No new matter has been introduced into the application as originally filed.

As originally filed, the sequence set forth in SEQ ID NO: 20 in the Sequence Listing actually reflected SEQ ID NO: 19 as indicated in the specification. In the amended Sequence

Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 20 reflects the sequence described as SEQ ID NO: 20 on page 96 of the specification (Example 10). No new matter has been introduced.

As originally filed, the sequence set forth in SEQ ID NO: 21 of the Sequence Listing actually reflected SEQ ID NO: 20 as indicated in the specification. In the amended Sequence Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 20 reflects the sequence described as SEQ ID NO: 20 in Example 10 of the specification. No new matter has been introduced.

As originally filed, the sequence set forth in SEQ ID NO: 22 of the Sequence Listing actually reflected SEQ ID NO: 21 as indicated in the specification. In the amended Sequence Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 21 reflects the sequence described as SEQ ID NO:21 in Example 10 of the specification.

As originally filed, the sequence set forth in SEQ ID NO: 23 of the Sequence Listing actually reflected SEQ ID NO: 22 as indicated in the specification. In the amended Sequence Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 22 reflects the sequence described as SEQ ID NO: 22 in Example 10 of the specification.

As originally filed, the sequence set forth in SEQ ID NO: 24 of the Sequence Listing actually reflected SEQ ID NO: 23 as indicated in the specification. In the amended Sequence Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 23 reflects the sequence described as SEQ ID NO: 23 in Example 10 of the specification.

As originally filed, the sequence set forth in SEQ ID NO: 25 of the Sequence Listing actually reflected SEQ ID NO: 24 as indicated in the specification. In the amended Sequence Listing provided on June 20, 2001 as well has herewith, SEQ ID NO: 24 reflects the sequence described as SEQ ID NO: 24 in Example 10 of the specification.

The nucleotide sequence AGG AGA AGA indicated on page 88, line 24 of the specification has now been included in the Sequence Listing. In addition, the specification has been amended to include the Sequence Identifier 48.

The sequences listed on page 78 of the specification are included in the Sequence Listing filed on June 20, 2001 and the Sequence Listing filed herewith. By this amendment, sequences listed on page 78 have now been given Sequence Identifiers SEQ ID Nos. 42-47. No new matter has been introduced.

The sequences listed on page 53 of the specification i.e., SEQ ID Nos. 34-39 are presently included in the Sequence Listing submitted herewith (and were also included in the previously filed Sequence Listing, June 20, 2001).

By this amendment, the sequences listed on page 104, lines 4 and 5, of the specification, have been given the Sequence Identifiers SEQ ID No:42 and SEQ ID NO:10, respectively.

Page 13, line 11 of the Sequence Listing has been amended to indicate that the sequence VRRRD/ExxxVEE has the Sequence Identifier SEQ ID NO:33.

SEQ ID NO:40 as submitted in the Sequence Listing June 20, 2001 has been deleted. The deleted sequence is presently set forth in SEQ ID NO:4.

SEQ ID NO:41 as submitted in the Sequence Listing submitted on June 20, 2001 is now renumbered as SEQ ID NO:40 and reflects the sequence set forth as clone FL67 depicted in Figure 1.

The sequences set forth as SEQ ID NOs: 42-48 as submitted on June 20, 2001 have now been renumbered as SEQ ID Nos: 41-47.

In view of the foregoing amendments to the specification, it is respectfully submitted that the present application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

Dated: July 25, 2001

Ann R. Pokalsky
Registration No. 34,697

Nixon Peabody LLP
990 Stewart Avenue
Garden City, New York 11530-4838
Telephone: (516) 832-7572
Facsimile: (516) 832-7555

ARP/mm

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)

I certify that the attached correspondence is being deposited on 7/25/01 with the U.S. Postal Service as first class mail under 37 C.F.R. § 1.8 and addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Maria J. Matos

Maria J. Matos

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Page 13, lines 7-16:

The *FL39* clone is 932 bp (SEQ ID NO:1) long and contains an ORF encoding a protein of 209 amino acids (SEQ ID NO:2) with a calculated molecular mass of 24 kDa. In its 3' UTR a polyadenylation signal can be [recognised] recognized. The amino-terminal part of the FL39 protein contains a repeated motif of 11 amino acids VRRRD/ExxxVEE (SEQ ID NO:33). This motif is not found in any other protein in the databanks and its significance is unknown. The FL39 protein also contains a putative nuclear localization signal (amino acids 23-26) and a PEST-rich region (amino acids 71-98; PESTFIND score +15.5) These sequences, rich in proline, glutamic acid, serine and proline, are characteristically present in ustable proteins (Rogers et al., 1986, Science 234, 364-368).

Page 78, second full paragraph:

Insertional amino acid sequence variants of a protein of the invention are those in which one or more amino acid residues are introduced into a predetermined site in said protein. Insertions can comprise amino-terminal and/or [carbocy-terminal] carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl [carbolyl] terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, $(histidine)_6$-tag, glutathione S-transferase, protein A, maltose-binding protein, dihydrofolate reductase, Tag • 100 epitope (EETARFQPQPGYRS) (SEQ ID NO:42), c-myc epitope (EQKLISEEDL)(SEQ ID NO:43), FLAG® -epitope (DYKDDDK) (SEQ ID NO:44), lacZ, CMP (calmodulin-binding peptide), HA epitope (YPYDVPDYA) (SEQ ID NO:45), protein C epitope (EDQVDPRLIDGK) (SEQ ID NO:46) and VSV epitope (YTDIEMNRLGK) (SEQ ID NO:47).

Page 88 line 16 to page 89, line 22:

The obtained *FL39* PCR fragment was purified, and cut with *NdeI* and *EcoRI* restriction enzymes. This fragment was cloned into the *NdeI* and *EcoRI* sites of pET derivative pRK172 (McLeod et al., 1987, EMBO J. 6, p729-736). The obtained *FL66* PCR fragment was purified, cut with *NcoI* and *BamHI* and cloned into the *NcoI* and *BamHI* sites of pET21d. FL66pET21d was transformed in *E. coli* BL21 (DE3). FL39pRK172 was co-

transformed in *E. coli* BL21 (DE3) with pSBETa (Schenk et al., 1995 Biotechniques 19, p 196-200). PSBETa encoded the tRNA ^{UCU} that is low abundant tRNA in *E. coli*, corresponding to codons AGG and AGA (arginine). Because of the presence of [a] an AGG AGA AGA sequence (SEQ ID NO:48) (Arg 5, Arg 6, Arg 7) at the beginning of *FL39* coding sequence, an increase of the tRNA ^{UCU} pool of *E. coli* is necessary for the translation of *FL39*. The FL66pET21d/BL21(DE3) and FL39pRK172, pSBETa/ BL21(DE3) *E. coli* recombinant strains were grown in LB medium, supplemented respectively with 50 µg/ml [ampicilline] ampicillin and 50 µg/ml [ampicilline] ampicillin; 25 µg/ml [kanamycine] kanamycin. The cells were grown at 37°C until the density of the culture reached an A_{600nm}=0.7. At this time point, 0.4 mM IPTG was added to induce the recombinant protein production. Cells were collected 3 hours later by centrifugation. The bacterial pellet from 250 ml culture was suspended in 10 ml lysis buffer (Tris.HCl pH7.5, 1 mM DTT, 1 mM EDTA, 1 mM PMSF and 0.1% Triton X-100) and submitted to three freeze/thaw cycles before sonication. Cell lysate was clarified by centrifugation 20 minutes at 8000 rpm. The pellet was collected, was suspended again in extraction buffer, the resulting suspension sonicated, and pellet collected by centrifugation 20 minutes at 8000 rpm. A third wash was performed the same way with Tris extraction buffer + 1M NaCl and a fourth wash with Tris extraction buffer. After the different washing steps, the pellet [contains] contained FL66 or FL39 protein at 90% homogeneity. The pellets were suspended in Laemli loading buffer (Laemmli, 1970, Nature 277, p 680-681) and FL66 and FL39 were further purified by SDS/ 12% polyacrylamide gel electrophoresis. The gel was stained in 0.025% coomassie brilliant blue R250 in water and destained in water. The strong band co-migrating at the 31 kDa molecular weight marker position was cut out of the gel with a scalpel. The polyacrylamide fragments containing FL66 or FL39 were lyopyhilized and reduced into powder. The rabbit immunization was performed in complete Freund [adjuvand] adjuvant, [sub-cutaneaous] sub-cutaneous, with [this] these antigen preparations. One injection [corresponds] corresponded to 100 µg of protein. The boosting [injection] injections were performed with non-complete Freund adjuvant, sub-cutaneous [sub-cutaneaous]. The obtained sera detected bands of the expected size in protein extracts prepared from 2-day-old actively dividing cell cultures. No signals were observed using the [pr-immune] pre-immune sera.

Page 104, lines 3-20:

The full length ICK2-coding region was amplified by polymerase chain reaction (PCR) using the 5'-AGACCATGGCGGCGGTTAGGAG-3' (SEQ ID NO:41) and

5'-GGCGGATCCCGTCTTCTTCATGGATTC-3' (SEQ ID NO:10) primers and the pFL39 plasmid as template, introducing NcoI and BamHI restriction sites. The amplified fragment was cut with NcoI and BamHI and cloned between the NcoI and BamHI sites of PH35S (Hemerly et al., 1995), resulting into the 35SFL39 vector. The CaMV35S/ICK2/NOS cassette was released by EcoRI and XbaI and cloned blunt into the SmaI site of PGSV4 (Heourt et al, 1994). The resulting vector PGSFL39, was mobilized by the helper plasmid pRK2013 into [Agrobacterium tumefaciens] *Agrobacterium tumefaciens* C58C1Rif^R harboring the plasmid pMP90. [A. thaliana] *A. thaliana* plants ecotype Col-O were transformed by the floral dip method (Clough and Bent, 1998). Transgenic plants were obtained on kanamycin-containing media and later transferred to soil for optimal seed production. For all analysis plants were grown [in vitro] *in vitro* with 16-h light/8-h dark illumination at 22° C on germination medium (GM, Valvekens *et al.*, 1988). Molecular analysis of the obtained transformants was performed by Northern as described by Jacqmard et al. (1999); and Western blotting and CDK kinase activity measurements as described by De Veylder et al. (1997).